Physiology and Endocrinology III

W187   Effects of ruminally digested and undigested snakeweed extracts on female Sprague-Dawley rats. R. A. Halalsheh,* D. M. Hallford, and T. T. Ross, *New Mexico State University, Las Cruces.*

Two studies were conducted to examine effects of snakeweed (SW) extracts (evaporate residues) on serum components and reproduction in female Sprague-Dawley rats. In Exp. 1, 36 rats at d 5 (d 1) of pregnancy were offered SW extracts (ethanol and hexane) at 20% and 30% of diet (25g/rat). In Exp. 2, 36 rats at d 5 (d 1) of pregnancy were offered ruminally digested SW extracted (ethanol and hexane) at 20% and 30% of diet (25g/rat). Each rat was assigned a non-SW control rat fed 5001 Rat Chow. Rats were fed for 10 d and BW was recorded on d 1 and 11. Blood samples were collected via heart puncture and rats were euthanized on d 11. Experimental design was completely random with split-plot when appropriate. In Exp. 1, rats consuming 20% ethanol SW extract (20% ESW) had decreased ($P < 0.05$) feed intake on d 1 to 3 and increased ($P < 0.05$) intake on d 10, while those fed the 20% hexane SW extract (20% HSW) had increased ($P < 0.05$) feed intake from d 6 through d 10 compared with control rats. Rats consuming 30% ESW resulted in decreased ($P < 0.05$) feed intake on d 1 to 3 and increased ($P < 0.05$) intake on d 8 to 10. Rats consuming 30% HSW decreased intake on d 1, 2, 3, and 5 and increased intake ($P < 0.05$) on d 7, 9, and 10 compared with controls. Rats consuming 20 and 30% HSW had increased ($P < 0.05$) aspartate and alanine aminotransferase concentrations. Blood urea nitrogen, albumin, and creatinine increased ($P < 0.05$) in treated rats compared with controls. In Exp. 2, rats consuming 20% ethanol extract of digested SW (20% EDSW), 20% hexane extract of digested SW (20% HDSW), and 30% ethanol extract of digested SW (30% EDSW) increased ($P < 0.05$) feed intake compared with controls and 30% HDSW decreased ($P < 0.05$) feed intake on d 1, 2, and 7. Alkaline phosphatase, BUN, globulin and creatinine increased ($P < 0.05$) in rats consuming SW extracts compared with controls. In exp 1 and 2, Serum P4 and number of pups were similar ($P > 0.05$) among treatments. Results indicate that SW, both ruminally digested and undigested may contain potential chemical compounds that cause a mild toxicity and ruminal digestion altered SW hepatotoxicity.

Key Words: snakeweed, Sprague-Dawley rats, toxicity

W188   Effect of niacin on heat shock protein gene expression in transformed bovine mammary epithelial cells. S. Rungruang,* J. L. Collier, and R. J. Collier, *University of Arizona, Tucson.*

Recent studies have indicated a niacin receptor, GPR109A, was differentially distributed in bovine liver, muscle, brain and fat tissues. Objectives of this study were to confirm presence of a niacin receptor in bovine tissues and to examine the effect of niacin on heat shock protein (HSP) gene expression in transformed bovine mammary epithelial cells (MAC-T). In this study,GPR109A was detected by agarose gel electrophoresis and quantified using quantitative real-time reverse transcription-PCR (q-PCR) in tissue samples from 3 cows. Tissues examined were mammary, uterine, ovary, liver, skin, cultured MAC-T and primary bovine mammary epithelial cells (BMEC). Ribosomal protein subunit 9 (RPS9) was used as the reference gene. The receptor was present in all tissues and cells. Expression of the gene for the receptor was highest in skin, the next highest was mammary ($P < 0.01$) and no difference was detected between liver, uterine and ovary receptor gene expression. The receptor in skin and mammary was 26.41 and 4.37-fold higher than liver, respectively ($P < 0.01$). The MAC-T were

treated with niacin in different doses (0, 0.01, 0.1 and 1.0 mM) and 2 incubation times (0 and 4 h) at 37 C before incubation in 42 C for 8 h. The expression of genes for HSP27 and HSP70 were analyzed by q-PCR. The expression of HSP27 and 70 genes was increased during heat stress ($P < 0.01$). The data indicated niacin had a dose effect on HSP27 ($P < 0.03$) and HSP70 ($P < 0.02$) expression. The analysis also identified a quadratic relationship in HSP27 ($P < 0.01$), and linear relationship in HSP70 ($P < 0.01$) gene expression. MAC-T supplemented with 0.1 mM niacin had the highest HSP27 and 70 gene expression during heat stress when compared with 0 mM (11.45 vs −2.41-fold, $P < 0.02$ and 77.73 vs 5.26-fold, $P < 0.01$, respectively).Longer incubation times with niacin increased HSP expression ($P < 0.01$). However, we did not detect an interaction between incubation time, dose and temperature. These data indicated that niacin increases HSP gene expression in bovine cells during heat stress.

Key Words: heat shock protein, bovine mammary epithelial cells, heat stress

W189   Effects of betaine on heat induced heat shock protein expression in primary bovine mammary epithelial cells. Y. Xiao*[1,2], J. L. Collier[1], S. Rungruang[1], L. W. Hall[1], F. R. Dunshea[3], and R. J. Collier[1], *[1]University of Arizona, Tucson, [2]Huazhong Agricultural University, Wuhan, Hubei, China, [3]The University of Melbourne, Parkville, Vic., Australia.*

Betaine, an organic osmolyte, is accumulated by cells and stabilizes protein structure and cellular metabolism during physiological stress. Decreased induction of heat shock protein-70 (HSP70) gene expression and anti-apoptotic effects were found in betaine-treated mammalian cell lines (Madin-Darby canine kidney and mouse embryonic fibroblast cells) exposed to a hypertonic environment. To evaluate effects of betaine on heat-shocked bovine mammary epithelial cells (BMEC), we isolated primary BMEC from mammary gland tissue of a pregnant Holstein cow, seeded in collagen for 6 d of culture then determined gene expression of HSP70 and HSP27 in cells treated with 0 mM or 25 mM betaine in thermoneutral (TN, 37°C) and heat stress (HS, 42°C) conditions at 0, 2, 4 and 8 h, using quantitative real-time PCR (qRT-PCR). Hypoxanthine phosphoribosyltransferase served as the reference gene. A one-way ANOVA was conducted on ΔC_t value with the PROC MIXED procedure of SAS. The fold change was calculated by the $2^{-\Delta\Delta C_t}$ method. After 8 h of HS exposure, morphology in 0 mM betaine-treated BMEC indicated distinct cell degradation, which was absent in 25 mM betaine-treated BMEC. The qRT-PCR indicated that, in TN conditions, expression of HSP70 was significantly ($P < 0.01$) decreased in 25 mM betaine-treated cells compared with 0 mM at 2 h and 4 h, and expression of HSP27 decreased significantly ($P < 0.01$) as well at 8 h. In HS condition, HSP70 expression peaked at 2 h in 25 mM betaine-treated BMEC and at 4 h in 0 mM. The expression of HSP70 in BMEC in control media was decreased significantly at 8 h compared with betaine-treated cells (20.80 ± 6.19 *vs.* 57.18 ± 6.19 fold, $P < 0.01$). A continuous increase of HSP27 expression was evident in all HS groups from 0 h to 8 h. Higher expression levels of HSP27 were detected in 25 mM betaine-treated BMEC than 0 mM at 4 h (21.93 ± 2.16 *vs.* 12.93 ± 2.16 fold, $P < 0.01$) and 8 h (61.55 ± 2.64 vs. 33.40 ± 2.64 fold, $P < 0.05$). Collectively, data indicated that betaine had a protective effect in heat-shocked BMEC by increasing expression of heat shock proteins during heat stress.

Key Words: mammary epithelial cells, betaine, heat shock protein

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W190   Cloning and responsiveness of bovine glucose-6-phosphatase promoter to cyclic AMP and glucocorticoids. Q. Zhang,* S. Koser, and S. Donkin, *Purdue University, West Lafayette, IN.*

Gluconeogenesis is an essential process in ruminants. Glucose-6-phosphatase (G6Pase) is a rate-limiting and final step in gluconeogenesis and catalyzes the release of glucose from liver. The objective of this experiment was to clone bovine G6Pase promoter and determine the effects of cyclic AMP (cAMP) and dexamethasone (DEX), on G6Pase promoter activity. Bovine genomic DNA was extracted from liver and used as a template to clone the promoter sequence. A region from −1012 to −17 bp relative to the transcription start site (TSS) and truncated sequences within this region, from −801 to −17 bp, −530 to −17 bp and −312 to −17 bp relative to the TSS were generated by PCR. Amplicons were sequenced and cloned into a eukaryotic expression vector (pGL-3 basic) and linked to a luciferase reporter gene. Each promoter-luciferase construct was transiently transfected into rat hepatoma (H4IIE) cells. Cells were treated with either a combination of 0.70 mM cAMP and 0.10 mM DEX or vehicle. Cells were harvested after 23 h and luciferase activity was determined in the cell homogenate. Basal luciferase activities for all 4 promoter sequences were not different from pGL-3 basic luciferase activity ($P > 0.05$). The activities of all G6Pase promoter sequences were induced ($P < 0.05$) with exposure to cAMP and DEX. The data demonstrate the direct role of cAMP and glucocorticoids in regulating bovine G6Pase expression through promoter activation. Furthermore, the data indicate responsiveness to cAMP and dexamethasone that is within the −312 to −17 bp region of the bovine G6Pase promoter.

Key Words: bovine glucose-6-phosphatase, promoter activity, cAMP and glucocorticoids

W191   Effects of heat stress on insulin production in β-TC-6 pancreatic cells. M. V. Sanz-Fernandez*[1], R. L. Boddicker[1], J. W. Ross[1], R. P. Rhoads[2], and L. H. Baumgard[1], *[1]Iowa State University, Ames, [2]Virginia Polytechnic Institute and State University, Blacksburg.*

Heat stress (HS) decreases feed intake but inexplicably increases plasma insulin and the insulin response to a glucose tolerance test in a variety of animal models. Whether HS directly or indirectly (via an altered hormonal or metabolic profile) alters insulin synthesis/secretion is not known. We utilized murine β-TC-6 pancreatic cells to directly evaluate the effects of HS on glucose stimulated insulin secretion (GSIS). Cells were exposed to 1 of 2 environments: 1) HS (41°C) or 2) thermo-neutral (TN; 37°C). After 2 h of environmental exposure, cells were incubated with 1 of 4 glucose concentrations: 1) 0 mM, 2) 2.5 mM, 3) 5 mM, and 4) 10 mM; and further exposed to their respective temperatures for an additional 2 h. Both HS and TN cells had a similar ($P > 0.10$) maximal GSIS at 2.5 mM glucose. To study the temporal pattern of HS effects on GSIS, β-cells were exposed to HS or TN environments for 0, 2, 6, 12, 24, 48 h; and incubated with either 0 or 2.5 mM glucose. HS decreased ($P < 0.01$) insulin production with time. By 48 h, HS β-cells had a 75% decrease ($P < 0.01$) in GSIS compared with TN cells. Taken together, our results demonstrate that HS decreases insulin secretion in β-TC-6 pancreatic cells. These data suggest that additional mechanisms participate to increase plasma insulin parameters during in vivo HS. This work was supported by USDA NIFA grant #2011–67003–30007.

Key Words: heat stress, insulin, pancreas

W192   Relationship of single nucleotide polymorphisms of the bovine NOS2 and NOS3 genes with disease resistance in feedlot steers. A. J. Davis,* D. L. Kreider, E. B. Kegley, J. T. Richeson, and D. L. Galloway, *Animal Science Department, University of Arkansas Division of Agriculture, Fayetteville.*

Nitric oxide synthase activity is a key element in the inflammatory process initiated by bovine respiratory disease (BRD). Single nucleotide polymorphisms (SNP) in the promoter region of the endothelial nitric oxide synthase (NOS3) and inducible nitric oxide synthase (NOS2) genes may be related to the etiology of BRD, as well as, the damage and pathology that occurs in the lungs as a result of the inflammatory process associated with BRD. Therefore the objective of this study was to characterize SNP in the promoter region of the NOS3 and NOS2 genes and to determine the relationship of SNP to disease resistance in feedlot calves. Steers were randomly selected from ongoing trials conducted at the University of Arkansas Stocker and Receiving Cattle Research Unit. Health and performance records were used to identify sick (n = 12) and healthy (n = 12) animals. Animals were defined as sick if pulled from their pen for treatment of respiratory disease or suspected respiratory disease. Healthy animals were defined as animals randomly selected from the same pen as the selected sick animals which did not display health related or performance related problems during the feeding period. Upon arrival to the facility, animals were weighed (avg BW = 256.583 ± 16.5 kg) and blood was collected via jugular venipuncture. DNA (n = 24) was extracted from EDTA treated whole blood and SNP identified by sequencing a 5′ region approximately 700 bp upstream from the start of exon 1 for the NOS2 and NOS3 gene promoter region. Eight SNP, 2 deletions, and 1 insertion were observed for NOS3 and 1 SNP for NOS2. Chi-squared analyses did not reveal interactions between SNP and sick feedlot calves ($P \geq 0.12$), although the number of animals used in this study was limited. Additional research is needed to determine the relationship of SNP of the bovine NOS2 and NOS3 genes with disease resistance.

Key Words: nitric oxide, single nucleotide polymorphism, bovine respiratory disease

W193   Hypothalamic and abomasal mRNA expression of regulatory feed intake genes in cows grazing different herbage allowances of native pastures. V. Bassaizteguy, A. Casal, A. L. Astessiano, A. Kaitazoff, M. Veyga, M. Carriquiry, and A. I. Trujillo,* *Universidad de la Republica. Facultad de Agronomia, Montevideo, Montevideo, Uruguay.*

In extensive rangeland cow-calf systems, annual variability of herbage allowance affects cow energy balance through changes in feed intake. There is scarce information about gene expression of key factors involved in the central and peripheral regulation of energy intake. Thus, the aim of this study was to evaluate the effect of 2 different herbage allowances of native pastures on hypothalamic (NPY, AgRP, POMC, CCK A receptor) and abomasal (ghrelin, CCK) mRNA expression. Pure and crossbred adult beef cows were used in a complete randomized block design and were maintained in the same herbage allowance throughout the year (2.5 vs. 4 kgDM/kgBW; LO vs. HI; n = 8, n = 5 respectively) since May 2007. Cows used in this study gestated and lactated one calf every year from 2007 to 2009. At the end of the third year, all cows were slaughter at 190 ± 15 d postpartum. Samples of hypothalamus and abomasum were collected to measure gene expression by SYBR-Green real time PCR using *RSP9* and β-actin as endogenous control genes. Data were analyzed using a mixed model that include the effect of herbage allowance as a fixed effect and cow genotype as a random effect. Means were considered to differ when $P < 0.05$. Expression of NPY and POMC

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mRNA did not differ due to herbage allowance. However, hypothalamic expression of AgRP mRNA tended ($P = 0.077$) to be less in HI than LO cows (0.84 vs. 2.3 ± 0.48) and CCK A receptor mRNA was numerically ($P = 0.157$) greater in HI than LO cows (1.91 vs. 1.31 ± 0.26). Herbage allowance did neither affect abomasal expression of CCK mRNA nor ghrelin mRNA expression. Results suggest that rangeland beef cows are sensitive to different nutritional planes and may respond by changing the expression of genes that regulate feed intake in hypothalamus.

Key Words: nutrition, feed intake regulation, mRNA

W194   Identification of short-chain fatty acid (SCFA) receptor transcripts in ruminal papillae and responses to SCFA infusion. K. Yuan,* L. K. Mamedova, S. H. Li, and B. J. Bradford, *Kansas State University, Manhattan.*

Large quantities of short-chain fatty acids (SCFA) are produced by microbial fermentation in the rumen, but little is known about the mechanisms underlying their regulatory effects in cattle. Recent studies identified SCFA, including acetate, propionate, and butyrate as ligands for G protein-coupled receptor 41 (GPR41) and 43 (GPR43); lactate as a ligand for GPR81; and β-hydroxybutyrate and possibly butyrate as the ligand(s) for GPR109A. The objective of this study was to evaluate the effects of SCFA infusions on the transcript abundance of GPR41, GPR43, GPR81, and GPR109A in ruminal papillae. Six ruminally cannulated lactating Holstein cows were randomly assigned to treatment sequence in replicated 3×3 Latin squares and fed a standard lactation diet. Initially, cows were infused with 10 mol/d sodium acetate, sodium propionate, or sodium butyrate for 2 d. However, during period (P) 1, both DMI and calculated energy intake were decreased by infusions ($P < 0.01$) relative to pre-treatment. Therefore, infusion rates were decreased to 5 mol/d for P 2 and P 3. Ruminal papillae were collected immediately after each infusion period, and the mRNA abundance of the GPRs were determined by quantitative RT-PCR relative to the internal control gene RPS9. Data were analyzed using the REML procedure of JMP. Results showed that GPR41, GPR43, GPR81 and GPR109A genes are expressed in ruminal papillae in dairy cows. Transcript abundance of GPR41, GPR43 and GPR109A were not altered by SCFA infusion types or rates ($P > 0.10$), but GPR81 expression was increased by 1.9 ± 0.28 fold ($P = 0.01$) following the 10 mol/d SCFA infusion rate compared with the 5 mol/d infusion (across infusates). Abundance of these transcripts were not correlated with DMI or total energy intake ($P > 0.10$). Interestingly, GPR41 mRNA was positively correlated with GPR43 ($P < 0.001$, $r^2 = 0.71$) and GPR81 ($P < 0.001$, $r^2 = 0.59$), and GPR43 was positively correlated with GPR81 ($P < 0.001$, $r^2 = 0.96$). This study verified the expression of these SCFA receptors in ruminal papillae and indicated possible co-regulation of gene expression across several GPRs. Further research is needed to examine the roles of these GPRs in mediating the regulatory effects of SCFA on rumen function in dairy cows.

Key Words: G protein coupled receptors, rumen, volatile fatty acid

W195   Calibration of a dynamic, mechanistic model of amino acid and insulin effects on protein synthesis in animal tissues to represent liver and skeletal muscle. E. R. El-Haroun[1,2], J. J. Kim*[1], D. P. Bureau[1], A. R. Willms[1], and J. P. Cant[1], *[1]University of Guelph, Guelph, Ontario, Canada, [2]Cairo University, Giza, Cairo, Egypt.*

The objective of the present study was to calibrate a dynamic, mechanistic model of nutritional control of protein synthesis in animal tissues to represent liver and muscle, respectively. The model predicts the fractional synthesis rate (FSR) of protein based on mass action

kinetics of translation initiation and elongation, and insulin- and amino acid-mediated regulation of the concentration of bound eukaryotic initiation factor-4E (c4eBp) and the recycling of eukaryotic initiation factor 2 (UF2d, F2d→F2t). Sensitivities of model predictions to 50% changes in each of 16 kinetic parameters were determined. Exponents in the initiation equation and the first-order rate constant for elongation had the greatest effect on tissue FSR and UF2d, F2d→F2t. The steady-state value of c4eBp was highly sensitive to all 5 parameters of factor-4E association and dissociation and lacked sensitivity to any of the other parameters. The strategy for calibration of the model to liver and muscle data was to fit the top 5 parameters to which c4eBp was most sensitive first, followed by fits of the top 5 parameters to which FSR was most sensitive. More than 99% of this error was attributed to random disturbance, indicating a lack of either slope or mean bias. Mean square prediction errors for c4eBp and FSR in liver were 32.9 and 5.0% of their observed means, respectively, with 56 and 99% of the respective errors attributed to random disturbance. Bias in c4eBp prediction was attributed to technical errors in its measurement and did not affect the accuracy or precision of FSR prediction. Sensitivity analysis and accuracy testing suggested that modification of the model structure was not required. Two unique sets of parameters were obtained to describe protein synthesis in liver and muscle in terms of the regulation of c4eBp by insulin and leucine, and of UF2d, F2d→F2t by uncharged tRNA.

Key Words: mechanistic model, amino acid, insulin

W196   Expression of adiponectin and leptin receptors and angiopoietin-like protein 4 (ANGLP4) mRNA differed in the of pure and crossbred beef cows grazing different herbage allowances of native pastures. M. Carriquiry,* M. Veyga, A. Casal, A. L. Astessiano, and J. Laporta, *School of Agronomy, UdelaR, Montevideo, Uruguay.*

Leptin, adiponectin, and ANGLP4 play important roles in energy expenditure, insulin sensitivity, glucose homeostasis, and lipid metabolism but information in ruminants is scarce. Therefore, the objective of this study was to evaluate the effect of the herbage allowance of native pastures and cow genotype, on hepatic expression of receptors of leptin (LEPRb), adiponectin (ADIPOR1 and ADIPOR2) and insulin (INSR), and ANGLP4 mRNA. Adult cows (n = 24) in a factorial arrangement of herbage allowances (2.5 vs. 4 kgDM/kgBW in average throughout the year; LO vs. HI) and cow genotype (purebred: Aberdeen Angus and Hereford vs. their F1 crossbred; PB vs. CB) were used in a complete randomized block design. Cows were maintained in the herbage allowance treatment since May 2007 and gestated and lactated one calf every year from 2007 to 2009. At the end of the third year, cows were slaughter at 190 ± 15 d postpartum and liver samples were collected to measure gene expression by SYBR-Green real time PCR using hypoxanthine phosphoribosyltransferase and β-actin as endogenous control genes. Data were analyzed using a mixed model and means were considered to differ when $P < 0.05$. Cow body weight and body condition score were greater in HI than LO cows and in CB than PB cows through the gestation-lactation cycle. At slaughter, hepatic ADIPOR2 and INSR mRNA did not differ among groups but ADIPOR1 mRNA tended ($P = 0.09$) to be greater and ANGLP4 mRNA was greater in CB than PB cows (1.21 vs. 1.62 ± 0.26 and 0.54 vs. 1.05 ± 0.27, respectively) and these differences were mainly due to cows in low herbage allowance. In addition, expression of LEPRb mRNA was affected by the interaction between herbage allowance and cow genotype, as its expression was highest in HI-PB, lowest in HI-CB and LO-CB, and intermediate in LO-PB cows (3.58, 1.58, 0.73, and 0.77 ± 0.21, for HI-PB, LO-PB, HI-CR, and LO-CB, respectively). Hepatic LEPRb, ADIPOR1 and ANGLP4 mRNA could play a role in adaptation

mechanisms to changes in energy balance through the production cycle of beef cows in rangeland conditions.

Key Words: adipokines, hepatic gene expression, beef cattle

W197   Gene expression analysis of glutathione peroxidase, catalase, and superoxide dismutase (Mn) in white blood cells from dairy cows receiving an apple base nutraceutic supplement. L. E. Escobedo-Morales, J. A. Grado-Ahuir,* C. Rodríguez-Muela, P. Hernández-Briano, and R. M. Villaseñor-González, *Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Chihuahua, Chihuahua, México.*

This work was carried out to analyze the gene expression of antioxidant enzymes in white blood cells of cows under a nutraceutical diet based on apple pomace. We studied 8 Holstein cows at fresh stage that were randomly assigned to one of 2 experimental groups, control (T) and supplemented with a nutraceutical feed (M), being both diets isolaloric. Blood samples were obtained from the caudal vein every 14 d for 2 mo. Ribonucleic acid from white blood cells was isolated and real time RT-PCR reactions were performed to measure expression levels of genes coding for superoxide dismutase (SOD2), catalase (CAT) and glutathione peroxidase (GPX) relative to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data were analyzed under a mixed model including treatment, period and their interaction as fixed effects and cow as a random effect. Expression level for CAT and SOD2 genes were higher ($P < 0.05$) in M than in T cows (1.40 and 1.43 times, respectively). There were no difference between groups ($P = 0.4893$) for the enzyme GPX. According to our results, it appears to be a relationship among the antioxidant effects of apple pomace with increased mRNA levels of 2 antioxidant enzymes CAT and SOD2 (mitochondrial), which may explain at the genetic level the synergy of its components to achieve the reduction of reactive oxygen species in dairy cows.

Table 1. Overall fold change in gene expression (least squares means ± standard errors) of genes for antioxidant enzymes

Gene	Apple pomace	Control
Catalase	2.17 ± 0.16[a]	1.55 ± 0.16[b]
Glutathione peroxidase	6.26 ± 0.43[a]	5.83 ± 0.43[a]
Superoxide dismutase 2	2.72 ± 0.19[a]	1.90 ± 0.19[b]

[a,b]Means with the same letter within a row are statistically similar (P < 0.05).

Key Words: apple pomace, antioxidant enzymes, dairy cows

W198   Measurements of saliva secretion and salivary fluxes of metabolites from jugular–arterial concentration differences, hemoglobin concentration, and jugular blood flow. A. C. Storm*[1], M. Larsen[1], and N. B. Kristensen[1,2], [1]*Aarhus University, Department of Animal Science, Tjele, Denmark,* [2]*Syddansskkvæg, Vojens, Denmark.*

The objective was to assess saliva secretion in lactating dairy cows during resting and rumination by measuring blood flow in right and left jugular vein and the concentration difference of hemoglobin in arterial and jugular blood. Three lactating dairy cows were surgically implanted with permanent catheter inserted into *A. intercostales dorsales* with the tip of the catheter placed in *Aorta*. On sampling days, temporary catheters were inserted 20 cm into *V. auricularis intermedialis* or *V. auricularis lateralis*, for infusion of the blood flow marker *p*-aminohippuric acid (11.17 ± 0.44 mmol/h), initiated 1 h before first blood sampling. Temporary catheters were also placed in the right and left *V. jugularis externa* for sampling of blood. Simultaneous blood samples from the artery and jugular veins were obtained during resting and rumination in 2 subsequent periods. For rumination and resting activity 2–4 and 2–3 repeated samples, respectively, were obtained with 5–10 min frequency. Cows were feed a 50:50 forages to concentrate diet. The difference between periods were dietary Na and K, (period 1 Na = 1.23, K = 1.41; period 2 Na = 0.37, K = 2.81% DM). Means within resting and rumination periods for each cow were analyzed statistically by a mixed model including main effect of activity (rumination and resting), period, and their interaction, considering cow as a random effect. The summed jugular blood flow, saliva secretion, and salivary flux of HCO_3^-, urea, and P were higher during rumination as compared with rest (Table 1), and were not affected by dietary Na or K. In conclusion, the current method for measuring saliva secretion produced reasonable estimates for secretion of selected metabolites in saliva.

Table 1. Jugular blood flow, salivary flow, and salivary flux of P, urea, and bicarbonate in lactating dairy cows during resting and rumination periods

Item	Rest	Rumination	SEM[1]	ACT[2]	Period
Jug. blood flow, L/h	199	430	22.7	<0.001	0.76
Saliva flow, L/h	7.4	13.6	0.77	<0.001	0.14
HCO_3^- salivary flux, mmol/h	870	1879	72	<0.001	0.07
P salivary flux, mmol/h	81	126	8.7	0.003	0.99
Urea salivary flux, mmol/h	22.7	37.8	3.17	0.011	0.13

[1]n = 3.
[2]ACT = rest or rumination.

Key Words: saliva secretion, flux

W199   Is colostrum quality in dairy cows related to postpartum health, production, or fertility? A. R. Dresch*[1], A. H. Souza[1], P. D. Carvalho[1], L. M. Vieira[1,2], J. L. M. Vasconcelos[3], R. A. Cerri[4], M. C. Wiltbank[1], and R. D. Shaver[1], [1]*University of Wisconsin-Madison, Madison,* [2]*University of Sao Paulo-VRA, SP, Brazil,* [3]*Sao Paulo State University Botucatu, SP, Brazil,* [4]*University of British Columbia, BC, Canada.*

Two studies were performed to investigate the relationship between colostrum quality and postpartum health, production and fertility in Holstein cows. In Experiment 1, colostrum samples were from 66 cows housed in a WI tie-stall barn. The DMI and milk yield measurements were daily and milk samples were collected from both AM/PM milkings twice weekly for determination of milk composition from calving to 70DIM. In Experiment 2, colostrum samples (n = 989) were from 2 commercial free-stall/parlor herds in WI. Colostrum was collected from the 1st milking and frozen for later quality analysis (Brix refractometer, 0 to 53% scale). Only cows with at least 3 weeks of dry period were used in the analysis. Data were analyzed using Proc Mixed and Proc Glimmix of SAS. Viscosity (r = 0.75, $P < 0.01$) and color (r = 0.46, $P < 0.01$) were highly correlated to Brix readings. Interestingly, there was no difference ($P > 0.10$) in proportion of primiparous (85%, n = 353) or multiparous (87%, n = 636) cows with Brix > 22%, that was considered to be good quality colostrum. In Experiment 1, cows with lower colostrum quality (LC, n = 18) were not different from cows with greater colostrum quality (GC, n = 48) for DMI, milk yield, fat%, or MUN. In contrast, LC cows tended ($P = 0.07$) to have greater linear SCC score in the first 5 weeks postpartum, with reduced milk protein content (2.9% vs 2.7%; $P < 0.01$) through 70DIM. In Experiment 2, although cows having retained placenta tended ($P = 0.07$) to have lower colostrum quality, but the logistic regression model indicated that cows with LC had similar %RP than GC, respectively (7% vs 4%, $P = 0.13$). In addition, LC cows were more likely to have postpartum

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metritis (13% vs. 7%, $P = 0.02$). Despite some significant associations between colostrum quality and RP and/or metritis, conception rate to 1st postpartum AI was not related to colostrum quality (LC = 33.8%, n = 133 vs GC = 34.2%, n = 856; $P = 0.90$). In conclusion, measurement of colostrum quality at calving was associated with some important production and health parameters postpartum; whereas, fertility following 1st postpartum AI was independent of colostrum quality. Supported by USDA Grant 2010–85122–20612.

Key Words: colostrum quality, postpartum health, dairy cow

W200   Effect of 17β-estradiol on cGMP-PK1 expression in myometrial longitudinal muscles. O. Y. Gulay*[1], A. Bulbul[2], M. S. Gulay[1], K. Altunbas[2], and O. O. Akkaya[2], *[1]Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Burdur, Turkiye, [2]Afyonkocatepe University, Faculty of Veterinary Medicine, Afyonkocatepe, Turkiye.*

The characteristics of spontaneous longitudinal myometrial contractility change due to direct actions of 17β-estradiol (ES) and nitric oxide (NO). NO inhibits the contractility of smooth muscles via guanylate cyclase (GC) where activated GC increases cGMP-PK1 expression and relaxes smooth muscles. Previous studies from our laboratories suggested that ES could inhibit the NO effect in myometrial longitudinal muscle via altering cGMP-PK1 expression. Thus, the aim of this study was to evaluate the effect of injecting different doses and time intervals of 17β-estradiol on cGMP-PK1 expression on myometrial longitudinal muscles of ovariectomized rats. Three to 6 mo old female Sprague Dawley rats (n = 71) were used in the current study. The ovariectomized rats (270 ± 20 g) were randomly assigned to one control (Ov) and 3 17β-estradiol injected groups of 18 rats each. Rats in the Ov group received daily sesame oil (0.2 mL, IM), whereas each rats in the 3 17β-estradiol injected groups were treated with daily 25, 50 and 100 µg estradiol in sesame oil (IM), respectively. Each group was further divided in 3 subgroups: 6 rats in each group were sacrificed by cervical dislocation at 18, 90 and 162 h. Uterus samples removed immediately after sacrifice for immunohistochemical evaluation (streptavidin-biotin-peroxidase method) to determine cGMP-PK1 expression in longitudinal muscles. For statistical evaluation of the data, one-way ANOVA was used. In myometrial longitudinal muscles, cGMP-PK1 scores (expression levels as mean $\pm$ SD) at 18, 90 and 162 h for Ov, 25, 50 and 100 µg estradiol groups were 7.33 ± 0.10, 7.41 ± 0.08, 7.00 ± 0.44, and 7.75 ± 0.11 ($P = 0.21$); 6.58 ± 0.53, 6.75 ± 0.11, 7.33 ± 0.27, and 6.50 ± 0.00 ($P = 0.24$); and 7.08 ± 0.08, 6.00 ± 0.89, 7.25 ± 0.11, and 6.16 ± 0.73 ($P = 0.34$), respectively. Our results indicated that effects of estrogen on cGMP-PK1 expression in uterine longitudinal smooth muscles were minimal. Thus, our data suggested that 17β-estradiol did not show its effect through cGMP-PK1 in uterine longitudinal muscles.

Key Words: ovariectomized rats, cGMP-PK1, estradiol

W201   Expression of sex steroid receptors in placental tissues during early pregnancy in sheep. L. P. Reynolds*[1], P. P. Borowicz[1], M. L. Johnson[1], J. Haring[1], R. Ashley[2], and A. T. Grazul-Bilska[1], *[1]Center for Nutrition and Pregnancy, Department of Animal Sciences, North Dakota State University, Fargo, [2]Department of Animal and Range Sciences, New Mexico State University, Las Cruces.*

Vascularization of the placenta during early pregnancy is critical for the successful establishment of pregnancy and also supports normal fetal growth and development. Sex steroids and their receptors are important regulators of angiogenesis and growth in reproductive tissues, including the placenta. However, little is known about the expression of sex steroid

receptors in placental tissues during early pregnancy. To examine mRNA expression of sex steroid receptors, we collected maternal (caruncular, CAR) and fetal (fetal membranes, FM) placental tissues on d14, 16, 18, 20, 22, 24, 26, 28, and 30 of early pregnancy (n = 5–6/day) and on d9–11 after estrus (nonpregnant [NP] controls; n = 5). Placental tissues were snap-frozen, RNA was extracted, and mRNA expression was evaluated using quantitative, real-time PCR. For both CAR and FM, nuclear progesterone receptor (P4R) mRNA was greatest ($P < 0.03$) in NP controls and through d22 of early pregnancy then declined and remained low on d24–30. For CAR, nuclear estrogen receptor (ER) α mRNA was greatest ($P < 0.01$) in NP controls and on d14 but declined on d18 of early pregnancy and remained low thereafter; for FM nuclear ERalpha was greatest ($P < 0.02$) on d20 of early pregnancy. For CAR, nuclear ERbeta mRNA was greater ($P < 0.01$) in NP controls than on any day of early pregnancy; for FM it did not change ($P = 0.33$) across days of early pregnancy. For CAR, membrane P4Ralpha and β were greatest ($P < 0.01–0.07$) in NP controls compared with any day of early pregnancy, whereas membrane P4Rgamma was 2- to 8-fold greater ($P < 0.01$) on d18 and 22 compared with NP controls or any other day of early pregnancy. For FM, membrane P4Ralpha, β, and gamma were elevated ($P < 0.01–0.10$) on d18 or 20 compared with NP controls or other days of early pregnancy. Thus, sex steroid receptors showed distinct patterns of expression in placental tissues during early pregnancy in ewes. These data establish the normal pattern of sex steroid receptor mRNA expression in maternal (CAR) and fetal (FM) placental tissues during early pregnancy, thus providing a basis for understanding the mechanisms by which sex steroids regulate placental growth and vascular development in normal and compromised pregnancies. Supported by USDA-NRI grant 2007–01215 to LPR and ATGB.

Key Words: placental angiogenesis, early pregnancy, sex steroid receptors

W202   Carryover effects on progesterone concentrations and fetal numbers in ewes given human chorionic gonadotropin. C. M. Richardson,* R. A. Halalsheh, D. M. Hallford, and T. T. Ross, *New Mexico State University, Las Cruces.*

Administration of hCG will increase serum progesterone (P4) and potentially increase number of lambs born. The objective of this study was to determine if a carryover effect of previous hCG administration would alter serum P4 concentrations and increase fetal number in ewes in subsequent years. A single dose of hCG was administered on d 4 post mating with the purpose to increase serum P4 concentrations in ewes and increase number of lambs born. Mixed aged Suffolk ewes (n = 40) received an intravaginal P4-containing insert (CIDR; 0.3 g P4) for 10 d to synchronize estrus. Ewes were mated with fertile rams on the first estrus after CIDR removal and were assigned to 1 of 3 treatments. Ewes that were treated with hCG (600 IU; i.m.) 1 year prior were divided into 2 groups; hCG/hCG and hCG/NO hCG. The hCG/hCG group received hCG (600 IU- i.m.) on d 4 post mating. Ewes that received hCG the year prior and were in the hCG/NO hCG group did not receive hCG and were administered a saline i.m. injection. Control ewes received saline both years. Jugular blood samples were taken from 7 ewes of each treatment group starting on d 1 through 21 d post mating to monitor serum P4. Ovulation rates were determined via laparoscopy on d 44. On approximately d 70, flank ultrasound was used to establish fetal numbers. A treatment x day interaction was observed for serum P4. Ewes in hCG/hCG group had greater ($P < 0.05$) P4 concentrations beginning on d 8 through d 15 than hCG/NO hCG and control treated ewes; whereas, treatments hCG/ NO hCG and controls had similar ($P > 0.05$) serum P4 concentrations. Ovulation rates, CL number ($P > 0.33$)

and fetal numbers ($P > 0.62$) did not differ among treatment groups. In conclusion, administration of hCG in subsequent years does not produce carryover effects allowing multiple year administrations of hCG.

Key Words: human chorionic gonadotropin, progesterone, fetal numbers

W203   Serum testosterone concentrations after feeding in rams treated with GnRH. M. M. Guardieiro*[1], F. L. M. Silva[1], A. A. Johnson[2], R. S. Gentil[1], P. L. J. Monteiro Jr.[1], D. M. Polizel[1], R. A. Souza[1], I. Susin[1], E. Oba[3], G. B. Mourão[1], and R. Sartori[1], *[1]University of São Paulo, Piracicaba, SP, Brazil, [2]Texas A&M University, College Station, [3]São Paulo State University, Botucatu, SP, Brazil.*

Circulating testosterone (T) is highly variable due to alterations in testicular T production, driven by pulses of luteinizing hormone, and alterations in T metabolism, primarily in the liver. In this study we attempted to develop an experiment to better understand the pattern of T release in male ruminants treated or not with GnRH and after feeding. The hypotheses for this study were: 1) circulating T will rapidly increase in rams following an LH pulse induced by exogenous GnRH treatment, 2) circulating T will decrease following feeding. Eight pubertal Santa Inês rams, weighing 56.6 kg on average, were used in a crossover design to test the first hypothesis and to provide initial observations related to the second hypothesis. Jugular blood samples were collected just before i.v. treatments that were performed 3 times a day (7:00, 11:00, and 15:00 h) with saline (Control group, n = 8) or GnRH (50 µg Gonadorelin, Fertagyl, MSD Saúde Animal; GnRH group, n = 8) and 30, 60, and 120 min after treatments. Rams were fed 1 h after the first i.v. treatment. Serum T concentrations were measured by radioimmunoassay. Data were analyzed using a generalized linear mixed model, considering the first-order heterogeneous autoregressive covariance matrix, due to repeated measures on the same ram at different hours. The model included the fixed effects of treatments, time of blood collection (hour) and treatment × hour interaction. Period and error were included as random effects. There was an effect of treatment ($P < 0.001$), hour ($P = 0.0123$) and treatment by hour interaction ($P < 0.001$). After the first GnRH treatment, T concentrations increased by 30 min with peak concentrations attained by 2 h after GnRH (8.8 ± 1.3 and 2.3 ± 1.3 ng/mL, GnRH vs. Control respectively; $P = 0.004$). There were also increased T concentrations after the second (0.5, 1, and 2 h) and third (1 and 2 h) GnRH treatments, although the peak T concentrations were lower compared with the first GnRH treatment. In the Control group, there was an apparent decrease in circulating T at 3 h after feeding with a subsequent increase in circulating T at 7 h after feeding. Thus, the first hypothesis on GnRH-induced LH pulses driving testicular T production was supported and future studies are needed to validly test whether time of feeding regulates circulating T, possibly through increased T metabolism. Supported by MSD Saúde Animal, FAPESP and CNPq of Brazil.

Key Words: sheep, testosterone, GnRH

W204   The potential effects of dietary nitrate on pregnancy mechanisms in ewes. K. J. Austin,* R. R. Cockrum, L. E. Speiser, and K. M. Cammack, *University of Wyoming, Laramie.*

Subacute levels of dietary nitrate have been implicated in embryonic loss, abortion and infertility in ruminants. Previous studies have not shown differences in the levels of progesterone or the number of lambs born to nitrate treated ewes compared with control ewes when nitrate was administered for 2 estrus cycles before breeding. However, nitrate toxicity is highly variable in sheep, and tolerance to such toxicity has been observed especially in cases of subacute, chronic exposure. Therefore, we hypothesize that chronic exposure to subacute levels of dietary nitrate may alter pregnancy mechanisms not observed with short-term exposures. Purebred Suffolk ewes (n = 24) were randomly assigned to one of 2 treatment groups, 0 mg/kg KNO_3 /BW (control) or 175 mg/kg KNO_3/ BW (nitrate treated) for 26 d. Ewes were weighed weekly and levels of KNO_3 adjusted according to BW. Ewes were synchronized, monitored for heat, administered KNO_3 for 2 full cycles, and mated to one of 2 intact rams. Ewes were bled daily for 26 d post breeding, then weekly until parturition. Blood was analyzed for progesterone (d 12–14, 50 and 80), *ISG15* and *MX2* mRNA (d 12–14) and circulating levels of PSBP (d 50 and 80). Progesterone levels did not differ in nitrate treated ewes compared with controls on d 12–14 ($P > 0.30$), d 50 ($P = 0.51$) or d 80 ($P = 0.92$) of pregnancy. Message for *MX2* did not differ on d 12 and 13 ($P = 0.24$) but tended to be upregulated (2.6 FC, $P = 0.07$) on d 14 of gestation in nitrate treated ewes compared with controls. Message for *ISG15* tended to be decreased on d 12 (2.3 FC, $P = 0.09$) and 13 (1.6 FC, $P = 0.13$) and upregulated on d 14 (3.8 FC, $P = 0.09$) of gestation in nitrate -treated compared with control ewes. Levels of PSPB in blood tended to be upregulated (1.6 FC, $P = 0.10$) on d 50 but did not differ between control and nitrate treated ewes on d 80 of pregnancy($P = 0.24$). Results imply that although no effects of dietary nitrate were observed immediately following short-term treatment, there may be chronic effects of nitrate in the diet of sheep which may impair the ewe's ability to maintain pregnancy. Perhaps ISG15 and MX2 play some role in protecting pregnancy against this challenge.

Key Words: nitrate, ISG15, MX2

W205   Effects of intravenous glucose infusion and nutritional balance on expression of enzymes responsible for catabolism of progesterone in cattle. F. Vieira[1], R. Cooke[2], A. Aboin[1], P. Lima[3], and J. L. Vasconcelos*[1], *[1]DPA-FMVZ-UNESP, Botucatu, SP, Brazil, [2]Oregon State University, Burns, [3]IBB-UNESP, Botucatu, SP, Brazil.*

The objective of this study was to evaluate the effects of glucose infusion and energy balance on serum concentrations of glucose, insulin, IGF-1, progesterone (P4), as well as mRNA expression of hepatic GHR1A, IGF-1, CYP2C and CYP3A in nonlactating, ovariectomized cows inserted with an intravaginal device P4 (CIDR). Fifteen Gir × Holstein cows were stratified by BW and BCS on d −28 of the study, and randomly assigned to: 1) negative nutrient balance (NB) and 2) positive nutrient balance (PB). Cows assigned to PB were supplemented individually once a day. From d −28 to −15 of the study, cows received a previously used CIDR, which was replaced by a new CIDR on d −14 and remained until the end of the study (d 1). On d 0, cows within nutritional treatment were removed from pastures and randomly assigned to receive, in a crossover design containing 2 periods of 24 h each (d 0 and 1): 1) intravenous glucose infusion (GLUC; 0.5 g of glucose/kg of BW, over a 3 h period), or 2) intravenous saline infusion (SAL; 0.9% NaCl, over a 3 h period). Cows were fasted for 12 h before infusions, and remained fasted during infusion and sample collections. Blood samples were collected at −12 (beginning of fasting), 0 (before infusion), 3 and 6 h after beginning of infusions via the coccygeal vein. Liver biopsies were performed at 0 and 3 h relative to beginning of infusions. Compared with PB cows, NB cows lost ($P < 0.05$) more BW (−23.1 vs. 16.5 kg) and BCS (−0.20 vs. 0.07). Cows receiving GLUC had greater glucose ($P < 0.01$) concentrations at 3 h relative to infusion compared with SAL cohorts (148.04 vs. 65.5 mg/dL). Cows receiving GLUC infusion had greater ($P < 0.05$) insulin concentrations compared with cows receiving SAL at 3 h, although this glucose-stimulated increase in insulin

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was greater in NB compared with PB cows (nutritional status x infusion x h interaction; 136.71 vs. 51.3 µUI/mL; $P < 0.01$). In PB cows, glucose infusion reduced the expression of GHR1A and CYP3A, but did not impact serum P4 concentration. In NB cows, glucose infusion increased expression of GHR1A and CYP3A and decreased serum P4 concentration. In conclusion, the effects of glucose infusion on serum insulin, P4, GHR1A and CYP3A mRNA expression were dependent on the cow nutritional status.

Key Words: insulin, CYP3A, progesterone

W206   Ex vivo model for endotoxic laminitis in ruminants. S. Schaumberger,* N. Reisinger, and G. Schatzmayr, *Biomin Research Center, Tulln, Austria.*

It is well known that laminitis in ruminants has a multifactorial etiology. As a key factor feeding of increased fermentable carbohydrate has been identified. If animals are not adapted to these rations, this may lead to rumen acidosis. Coincident with the change in the rumen pH is the release of endotoxin from gram-negative bacteria. An enhanced absorption of bacteria, endotoxin, lactic acid and histamine through the rumen, leads to a direct or indirect disruption in the micro-circulation of the corium and later to the lesions observed in laminitis. Objective of our ex vivo study was to get a better understanding of the direct influence of endotoxins on the lamellar structure of the claw and the separation from the coffin bone. Four normal claws were collected from the slaughter housed. Claw explants consisted of 2 mm of the inner claw wall, 6 intact epidermal lamellae and 2 mm of connective tissue. Explants were cultured with 1 mL culture medium and in 0.9% sodium chloride solution at 37°C and 5% CO2. After 24 and 48 h, explants were studied for lamellar separation. In a second experiment, explants were cultured with different concentrations (200 to 5 µg/mL) of lipopolysaccharides (LPS) from *Escherichia coli* O55:B5 for 24 and 48 h. Explants cultured only in medium were used as a negative control, and those cultured in 0.9% sodium chloride solution as a positive control. After incubation explants were tested for their integrity. Viability was tested with the water soluble tetrazolium (WST)—1 assay. Explants were considered as viable if absorbance of the medium containing 1% WST reagent increased after 2 h of incubation. All explants cultured only in medium for 24 and 48 h remained intact, and all cultured in 0.9% sodium chloride solution separated. Down to 20 µg LPS/mL 100% of the explants seperated. From 10 µg to 5 µg LPS/mL, separation of 33% and 66% of the explants could be detected. Explants still were viable after 24 and 48 h of incubation. Endotoxins of *E. coli* showed a concentration dependent lamellar separation in claw explants. This model may be used to investigate substances which affect the development of bovine laminitis. Further experiments will be carried out to investigate supernatants and explants for their cytokine activity to explain the inflammation process induced by LPS.

Key Words: ruminants, laminitis, endotoxin

W207   Effect of different centrifugation protocols and comparison of four extenders for storage of cooled Caspian horse spermatozoa for 48 hours. H. Nouri[1], A. Towhidi*[1], and M. Bahreini[2], [1]*Department of Animal Science, Faculty College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran,* [2]*Animal Breeding Center of Iran (A.I. lab), Iran.*

The use of cooled transported equine semen continues to gain popularity among breeders as its acceptance increases among breed registries. Two experiments were conducted to evaluate the impact of 4 extenders and different centrifugation protocols on cooled preservation of Caspian horse semen. In experiment 1, extenders tested included EZ-mixin (Minitube, Germany), Kenney's (Minitube, Germany), INRA96 (IMV, France), and CPE (Caspian horse extender). Semen was collected from 4 adult Caspian horse with artificial vagina and each ejaculate was divided and extended in each of the aforementioned extenders and stored at 4ËšC. Motility measures were determined using computer-assisted sperm analysis at 0, 24, and 48, hours after collection. Samples were evaluated for total motility, progressive motility (PM), straight-line velocity, curvilinear velocity, straight-line distance. Total motility and PM decreased over time in storage ($P < 0.05$). Sperm stored in INRA 96, and CPE retained the most total motility and PM over the 48-h period ($P < 0.05$). CPE had the highest measurements for curvilinear velocity, straight line velocity, and curvilinear distance ($P < 0.05$). In experiment 2: The objectives of this experiment were to determine the effects of centrifugation on Caspian horse sperm progressive motility, viability, and plasma membrane integrity. Ejaculates from 4 Caspian horses were collected, extended (CPE) to a concentration of 50×10^6 cells/mL, and subjected for 4 and 7 min to: 1) no centrifugation (NC); 2) $500 \times g$ (500); 3) $1000 \times g$ (1000); and 4) $2000 \times g$ (2000). Before and after centrifugation (d 0), and after 24 and 48 h of cooling, sperm motility was assessed by computer assisted semen analysis, and samples were stained with Eozin negrosin for viability, and with Hypo-osmolarity swelling test (Host) for membrane integrity. Compared with the other treatment groups the 2000 treatment group showed reduced motility, viability, and membrane integrity ($P < 0.05$). The 500 and 1000 treatment groups centrifugation of semen Compared with uncentrifuged samples resulted in a better sperm quality after chilled storage. The 500 and 1000 treatment groups yielded lower recovery rates than the 2000 treatment group ($P < 0.05$). Centrifugation at $1000 \times g$ for 7 min did not damage Caspian horse sperm.

Key Words: Caspian horse, centrifuge, extenders

W208   Pigs fed camelina meal increases liver CYP8B1 expression. W. J. Meadus*[1], P. Duff[1], T. McDonald[2], and W. Caine[1], [1]*AAFC-Lacombe, Lacombe, AB, Canada,* [2]*Olds College, Olds, AB, Canada.*

Camelina, also known as false flax, has commercial potential, as an oil seed crop for biofuels and biolubricants grown on marginal lands. Camelina seed has an oil content of >40% and this oil is high in n-3 fatty acids and γ-tocopherol. The oil is not approved for human consumption because as a member of the mustard family (Brassicaceae) and is suspected to contain high levels of erucic acid (C22:1 n-9) and glucoinolates. Camelina meal is the by-product after the oil has been extracted. Pigs (n = 26) were fed 3.7% and 7.4% camelina meal for 20 d starting at weights of 12 kg and finished at 17kg. The livers of the pigs were obtained at slaughter and immediately extracted for total RNA. Total RNA was examined for gene expression changes by microarray analysis using the Rat drug metabolism: phase 1 array and the Human Drug Metabolism: phase 2 enzyme arrays, of SAbioscience. Expression analysis (ΔCt ref-sample) on the rat array identified the cytochrome P450 family 8b1 (Cyp8b1) and aldehyde dehydrogenase 2 (Aldh2); and on the human array, glutathione S-transferase mu 5 (GSTM5), and thiosulfate sulfurtransferase (TST), as being significantly upregulated, relative to the control livers. The porcine version of Cyp8b1 was cloned and confirmed, for significant ($P < 0.05$) upregulation of ~4-fold, by real-time PCR. The Cyp8b1 is associated with hyocholic bile acid formation. The camelina meal must be inducing bile acid formation, possibly due to erucic acids.

Key Words: camelina, gene expression, Cyp8b1

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W209   Effect of exogenous testosterone on testes characteristics of large white pigs in a humid enviroment. A. O. Ladokun*[1], J. R. Otite[2], O. M. Alabi[3], and D. O. Adejumo[2], *[1]University of Agriculture, Abeokuta, Ogun, Nigeria, [2]University of Ibadan, Ibadan, Oyo, Nigeria, [3]Bowen University, Iwo, Osun, Nigeria.*

A total of 24 pigs were used for the experiment to determine the effect of exogenous testosterone (testosterone enanthate) administered by intramuscular injection on testicular characteristics especially daily sperm production (DSP). Pigs were treated once weekly from birth until 24 weeks of age with vehicle or testosterone enanthate. Testosterone enanthate was given at the rate of 1.2mg/kg body weight. Corn oil was used as the vehicle. At the end of the 24 weeks, the pigs were slaughtered and their testes harvested. The parameters measured include seminiferous tubule diameter, paired testes weight (PTW), testis density, daily sperm production (DSP) from gonadal sperm reserves (GSR) and DSP from quantitative testicular histology (QTH). Data obtained were analyzed using Complete randomized design (one-way ANOVA) of Statistic Analytical System (SAS) package 2001. The results show that testosterone enanthate significantly ($P < 0.05$) increased the values of DSP from QTH (18.12 ± 3.00; 35.28 ± 4.11). The same trend was recorded for Seminiferous tubule diameter ($117.73 \pm 9.10\mu$ and $148.29 \pm 8.70\mu$ respectively for control and testosterone). This present results show that exogenous administration of testosterone to large white male pigs, could be used to accelerate spermatogenesis and thus reproductive efficiency in swine herds especially for boars.

Table 1. Testicular characteristics of boars administered testosterone enanthate

Testicular characteristics	Control (blank injection)	Testosterone enanthate
Paired testes weight-PTW g	101.46[b]	220.43[a]
PTW per kg bodyweight	3.27[b]	3.93[a]
Testes density	1.10	1.06
DSP from GSR (x109)	4.37[b]	8.56[a]
DSP from QTH (x106)	18.12[b]	35.28[a]
Seminiferous tubule diameter (μ)	117.73[b]	148.29[a]
DSP per unit tubule diameter	28.12[b]	50.16[a]
Volume % of round spermatid nuclei	1.74[b]	3.78[a]

[abc]Means in the same row with different superscripts differ significantly ($P < 0.05$).

Key Words: exogenous, testosterone, testes

W210   Effect of heat stress on phosphatidylinositol-3 kinase signaling in gilt ovaries. J. Nteeba,* E. E. Ullerich, S. C. Pearce, R. Boddicker, J. W. Ross, L. H. Baumgard, and A. F. Keating, *Department of Animal Science, Iowa State University of Science and Technology, Ames.*

Heat stress (HS) negatively affects reproductive performance in swine, but the biological reasons responsible for this impaired fecundity are poorly understood. Paradoxically HS decreases feed intake but unexplainably increases plasma insulin in a variety of animal models including pigs. Insulin influences ovarian phosphatidylinositol-3 kinase (PI3K) signaling, which is important for follicle viability and regulating follicle activation and steroidogenesis. Two downstream mediators of PI3K action are Protein kinase B subunit 1 (*Akt1*), and the forkhead transcription factor subunit 3a (*Foxo3a*). This study investigated the effects of HS on PI3K signaling in the porcine ovary. Crossbred gilts (35 ± 4 kg) housed in constant climate controlled rooms in individual pens with ad libitum feed intake were exposed to thermal neutral (TN)

conditions (20°C; 35–50% humidity; n = 3–6) or HS conditions (35°C; 20–35% humidity; n = 3–6) for 7 or 35 d to simulate acute and chronic HS, respectively. Gilts were euthanized, one ovary was stored at −80°C and the other ovary was fixed in 4% paraformaldehyde. Total RNA was isolated and levels of *Akt1* and *Foxo3a* mRNA were quantified by RT-PCR using *GAPDH*, as the internal control gene. The insulin receptor (IR) and phosphorylated (active) AKT (pAKT) proteins were localized by immunofluorescence staining. On d 7, HS decreased (12-fold; $P < 0.05$) mRNA encoding *Akt1*, but environment had no effect on *Foxo3a* mRNA abundance. After 35 d and relative to TN controls, HS increased ($P < 0.05$) *Akt1* (2.0-fold) and *Foxo3a* (2.1-fold) mRNA. Oocyte cytoplasm and cytoplasmic membrane of all stage follicles stained positive for the IR protein. pAKT protein was located in the oocyte cytoplasm of all stage follicles, with apparent greater expression in larger stage follicles. Additionally, theca and granulosa cells of pre-ovulatory follicles were positive for pAKT protein. These data suggest HS leads to altered expression of PI3K signaling pathway members, which could alter dynamics of follicle activation, affect follicle viability and potentially alter ovarian steroid synthesis, thus leading to negative effects on fertility in swine.

Key Words: heat stress/hyperthermia, reproductive physiology, swine/porcine/pig

W211   Acute duration heat stress alters expression of cellular bioenergetic-associated genes in skeletal muscle of growing pigs. S. G. L. Won*[3], G. Xie[3], R. L. Boddicker[1], J. N. Rhoades[2], T. L. Scheffler[3], J. M. Scheffler[3], M. C. Lucy[2], T. J. Safranski[2], J. T. Selsby[1], S. Lonergan[1], L. H. Baumgard[1], J. W. Ross[1], and R. P. Rhoads[3], *[1]Iowa State University, Ames, [2]University of Missouri, Columbia, [3]Virginia Polytechnic Institute and State University, Blacksburg.*

Heat stress (HS) causes significant losses to the US swine industry in several production and health areas including efficient lean tissue accretion. Perturbations in skeletal muscle metabolism may participate in this defect. The study objectives were to examine the expression of genes related to cellular bioenergetics in skeletal muscle of piglets subjected to thermal stress in utero and/or during postnatal life. To accomplish this, 48 offspring from 14 first parity gilts were prenatally exposed to 1 of 4 environmental treatments (TNTN, TNHS, HSTN, or HSHS). TNTN and HSHS sows were exposed to thermal neutral (TN, cyclical 18–22°C) or HS conditions (cyclical 28–34°C) for all of gestation, respectively. HSTN and TNHS treatments represent sows heat-stressed for the first or second half of gestation, respectively. At 12 weeks of age, offspring were exposed to one of 2 postnatal thermal environments for 24 h: constant TN (21°C) or HS (35°C). Gestational treatments were randomized across postnatal treatments, with each sow represented equally between the 2 postnatal treatments. Pigs were sacrificed after the 24-h TN or HS treatment and longissimus dorsi skeletal muscle samples collected for total RNA isolation. Real-time PCR was used to measure gene expression of pyruvate dehydrogenase kinase 4 (PDK4), hexokinase 2 (HK2), peroxisome proliferative activated receptor, gamma, coactivator 1 α (PGC-1α), mitochondrial transcription factor A (TFAM), succinate dehydrogenase complex (SDHA), cytochrome C oxidase (Cox5B), and Cytochrome *b* (Cyt B). Data was normalized using eukaryotic translation elongation factor 1 α 1 (EEF1A1) as a control gene and analyzed by the Proc Mixed procedure in SAS. Data analyses did not indicate interactions between gestational and postnatal treatments for the genes studied. Only Cyt B gene expression was altered by gestational HS exposure with HSHS increasing expression up to 2-fold over TNHS, HSTN and TNTN ($P < 0.01$). Neither in utero nor postnatal HS altered Cox5B or PGC-1α mRNA abundance. Postnatal HS significantly increased HK2 by 25%

($P < 0.05$) and tended to increase PDK4 8-fold ($P = 0.076$). In contrast, TFAM, SDHA, and Cyt B gene expression decreased significantly during postnatal HS ($P < 0.05$). These data indicate that acute postnatal HS alters skeletal muscle bioenergetic gene expression, which may favor enhanced glycolysis and a reduction in mitochondrial respiration. This work was supported by USDA NIFA grant #2011–67003–30007.

Key Words: heat stress, skeletal muscle, metabolism

W212   Effect of heat stress (HS) on thermal regulation during pregnancy in first parity sows. M. C. Lucy*[1], T. J. Safranski[1], J. N. Rhoades[1], J. W. Ross[2], N. K. Gabler[2], R. P. Rhoades[3], and L. H. Baumgard[2], [1]*University of Missouri, Columbia, [2]Iowa State University, Ames, [3]Virginia Tech, Blacksburg.*

First parity sows suffer from seasonal infertility that is associated with HS. The objective was to test the effects of HS during gestation and assess carryover effects after farrowing in first parity sows. Nulliparous gilts (n = 23) were brought into environmental chambers, inseminated and assigned to one of 4 ambient temperature treatments [TRT; HS (28 to 34°C; relative humidity, RH 80 to 55%) or TN (18 to 22°C; RH 70 to 60%)] that were applied for either the entire gestation [HSHS (n = 4) and TNTN (n = 4)] or for the first (wk 2 to 8) or second (wk 9 to 15) half of gestation [TNHS (n = 4) or HSTN (n = 4)]. There were 3 HSTN and 4 TNHS gilts that were not pregnant after insemination. Rectal, ear and shoulder temperatures (RT, ET, and ST) and respiration rate [RR; breaths per minute (BPM)] were collected twice daily (0900 and 1500h). There was an effect of wk on RT because, regardless of TRT, the RT decreased ($P < 0.001$) in pregnant sows (38.5 ± 0.1 to 38.3 ± 0.1°C; wk 2 to 15). The decrease in RT was not observed in non-pregnant sows (38.4 ± 0.1 to 38.6 ± 0.1°C; wk 2 to 15). There was a TRT by wk interaction for RT because the effects of HS (HSHS, HSTN, TNHS, and TNTN, respectively) were greatest during early gestation (38.4 ± 0.1, 38.5 ± 0.1, 38.2 ± 0.1, 38.3 ± 0.1°C for wk 5), were less in mid-gestation (38.3 ± 0.1, 38.2 ± 0.1, 38.2 ± 0.1, and 38.3 ± 0.1 for wk 10) and increased in late gestation (38.4 ± 0.1, 38.2 ± 0.1, 38.3 ± 0.1, and 38.2 ± 0.1 for wk 15). Effects of HS on RR were greatest during late gestation (53 ± 3, 28 ± 3, 81 ± 3, and 23 ± 3 BPM for wk 15). Changes in ET and ST ($P < 0.001$) were associated with changes in room temperature. Sows moved to a farrowing room (22°C) during the last wk of gestation. The RT, ET, and ST (respectively) increased ($P < 0.001$; 38.3 ± 0.1 to 39.5 ± 0.1°C, 33.1 ± 0.2 to 36.6 ± 0.2°C, 32.1 ± 0.2 to 35.5 ± 0.2°C; d −5 to d 10) after farrowing (during lactation) but there were no carry-over effects of TRT. In summary, sows decreased RT during gestation. The ET, ST, and RR responded to HS. Changes in RT in response to HS were small during gestation. Lactation under non-HS conditions (22°C) was associated with large changes with RT, ET, and ST. This project was supported by USDA NIFA 2011–67003–30007.

Key Words: sow, heat stress

W213   Hair cortisol concentrations—Influence of color and location in Holstein cows. R. L. A. Cerri*[1], A. M. Tabmasbi[2], and D. M. Veira[3], [1]*Land and Food Systems, University of British Columbia, Vancouver, BC, Canada, [2]Fedowsi University of Mashhad, Mashhad, Iran, [3]Agriculture & Agri-Food Canada, Agassiz, BC, Canada.*

Cortisol is often used to measure stress. A change in blood cortisol after exposure to a stressor gives an estimate of an acute stress response. However, the episodic daily fluctuations make blood measures of cortisol unsuitable for measuring chronic stress. Cortisol levels within milk or feces reflect circulating levels of cortisol over a longer period than a single blood sample, but still is less than ideal for assessing chronic stress. Recent research in humans and wildlife has shown significant correlations between hair cortisol levels and ill health. Our objective was to determine the potential of extracting and measuring cortisol in hair from cattle and whether its location and color influence cortisol concentrations. We measured the level of cortisol in black and white hair of Holstein cows (n = 18). Cows had both black and white hair shaved from the shoulder, top line and hip; white hair was also harvested from the tip of the tail. The hair samples were cleaned of dirt and dander, washed in water then with isopropanol, dried and then ground in a ball mill or finely cut with scissors. Cortisol was extracted with methanol before being measured using an ELISA kit. Data was analyzed by ANOVA using SAS (significant if $P < 0.05$). Cortisol concentration was similar between locations; however, the white hair had higher ($P < 0.001$) levels than black hair 13.7 ± 1.01 vs. 6.8 ± 1.63 pg/mg, respectively. Primiparous cows had higher ($P < 0.001$) cortisol levels than older cows 21.9 ± 1.80 and 4.37 ± 1.48 pg/mg. Samples processed with a ball mill had higher ($P < 0.006$) cortisol levels than those cut up with scissors 12.6 ± 1.03 vs. 7.9 ± 1.61 pg/mg. Hair from the tail of the Holstein cow is always white and had numerically higher cortisol levels than hair from other locations. It also regrows more rapidly than other sites, allowing sampling as frequently as every 3 wk making it suitable for measuring chronic cortisol levels over extended periods of time. This approach is being used to monitor cortisol levels during lactation and its relationship to health and reproduction.

Key Words: cortisol, hair, cows

W214   Animal and ovarian parameters affect fertilization and embryo quality in high-producing lactating dairy cows. R. L. A. Cerri*[1], W. W. Thatcher[2], and J. E. P. Santos[2], [1]*University of British Columbia, Vancouver, BC, Canada, [2]University of Florida, Gainesville.*

A total of 418 oocytes-embryos from single ovulating lactating Holstein cows were recovered 6 d after artificial insemination (AI). Cows were enrolled in a presynch-ovsynch program starting at 30 ± 3 d in milk. Ovarian responses were evaluated by ultrasonography and blood was analyzed for progesterone (P4) throughout the study. The body condition score (BCS) was measured at enrollment and uterine flush. Responses related to embryo quality were evaluated (tendency $P < 0.10$; significant $P < 0.05$) against parity (primiparous vs multiparous), cyclicity (until 44 ± 3 d in milk), BCS at enrollment (Low < 2.75 > Moderate), BCS change between measurements, ovulation to first GnRH of ovsynch, follicle size at AI (Small < 18mm > Large), and concentrations of progesterone (P4) at PGF (Low < 3 ng/mL > High), AI (Low < 0.2 ng/mL > High) and 6 d after AI (Low < 2 ng/mL > High). Moderate BCS tended to improve embryo quality by increasing proportion of grades 1–2 (73.1 vs 62.4%) and transferable embryos (84.6 vs 77.7%) and decreasing degenerated embryos (22.3 vs 15.4%). Ovulation to the first GnRH of the synchronization protocol improved proportion of embryos grades 1–2, transferable embryos, and live blastomeres, whereas decreased degenerated and unfertilized oocytes-embryos. Large follicles at AI increased fertilization rate (86.5 vs 78.2%) and the mean number of accessory sperm cells attached to the zona pellucida (28.1 ± 3.8 vs 17.8 ± 4.5). High P4 at PGF increased number of blastomeres in embryos (33.4 ± 2.6 vs 26.4 ± 2.3). Low P4 at AI tended to increase fertilization rate (88.0 vs 79.6%), oocyte-embryos grades 1–2 (60.9 vs 52.5%), mean number of cells (35.0 ± 2.3 vs 24.8 ± 2.5) and percentage of live cells (94.7 vs 88.9%), whereas it decreased degenerated and unfertilized oocytes-embryos (36.4 vs 27.1%). High P4 6d after AI significantly decreased proportion of transferable (76.9 vs 83.6%) and increased

degenerated embryos (23.1 vs 16.4%). In conclusion, moderate BCS, large follicles with restricted length from emergence to ovulation, and very low P4 levels at AI are associated with greater fertilization rate and embryo quality.

Key Words: embryo quality, fertilization, ovarian dynamics

W215   Relationships between sperm motility and in vivo and in vitro fertility of Holstein and Jersey bulls. M. D. Utt*[1], M. A. Coutinho da Silva[2], C. A. Messerschmidt[2], J. M. DeJarnette[3], C. E. Marshall[3], F. A. Abreu[1], and M. L. Day[1], [1]Department of Animal Sciences, The Ohio State University, Columbus, [2]Department of Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, [3]Select Sires Inc., Plain City, OH.

Sperm motility has been correlated to bull fertility in the past. Within the last 20 yr, computer-assisted sperm analysis (CASA) has provided a more detailed and objective evaluation of sperm motility. The objective of this experiment was to determine correlations of post-thaw CASA motility data to the following fertility estimates: sire conception rate (SCR; USDA, 2008), heterospermic in vivo (H-VIVO*) and in vitro (H-VITRO) competitive indices, and in vitro embryo cleavage rate (CLV). Extended semen from Holstein (H, n = 3) and Jersey (J, n = 3) bulls was either frozen homospermically or total sperm mixed 1:1 (H:J) to create 9 H/J heterospermic pairings that were later used for in vivo AI (H-VIVO*) or in vitro fertilization (H-VITRO). Frozen/thawed homospermic aliquots were used for determination of CLV or sperm motility parameters by CASA [n = 4 straws per bull; total motility (TM), progressive motility (PM), average path velocity (VAP), straight line velocity (VSL), amplitude of lateral head movement (ALH), tail beat frequency (BCF), and straightness (STR) and linearity of path (LIN)]. In vitro fertilization was performed on approximately 140 or 180 cumulus-oocyte complexes for each for each bull (CLV) or H/J pair (H-VITRO). Competitive indices were calculated for each bull based on mean proportion of calves or cleaved embryos sired by each bull across pairings (21 to 31 random parentage tests per pair; H-VIVO or H-VITRO). SCR data (0.0, 0.0, 0.0, +0.3, +2.0, +2.0) for the 6 bulls were not correlated to any sperm motility parameters. PM was correlated (r = 0.92; $P < 0.05$) only to H-VIVO. LIN, STR, and BCF were positively correlated to H-VIVO (r = 0.87, $P < 0.05$; r = 0.93, $P < 0.05$; and r = 0.78, $P < 0.10$, respectively) but negatively correlated to CLV (r = - 0.82, $P < 0.05$; r = - 0.83, $P < 0.05$; and r = - 0.75, $P < 0.10$, respectively). ALH was correlated to H-VITRO (r = 0.74, $P < 0.10$) and CLV (r = 0.73, $P < 0.10$) but not H-VIVO. In conclusion, correlations of CASA data to fertility differed between both heterospermic methods and between homo- and heterospermic methods in vitro. *Kasimanickam et al., 2006, 2007

Key Words: bull, fertility, sperm

W216   Placement of semen in uterine horns failed to improve fertilization rates in superovulated Holstein cows. P. D. Carvalho*[1], A. H. Souza[1], A. R. Dresch[1], L. M. Vieira[1,2], K. S. Hackbart[1], D. Luchini[3], S. Bertics[1], N. Betzold[4], M. C. Wiltbank[1], and R. D. Shaver[1], [1]University of Wisconsin-Madison, Madsion, [2]University of Sao Paulo-VRA, SP 05508, Brazil, [3]Adisseo, Alpharetta, GA, [4]U.S. Dairy Forage Research Farm, Prairie du Sac, WI.

This experiment was designed to test whether depositing the sperm in the uterine horns could improve fertilization rates in superovulated cows with synchronized ovulations. Holstein cows (n = 72), were milked twice daily and housed and fed individually in tie-stalls. Animals were blocked by parity and calving dates and randomly assigned to one of 2

treatments: 1) Semen placed in the uterine body (BAI); and 2) Semen placed in the greatest curvature of the uterine horns (HAI; semen dose split between the 2 horns). All cows had a synchronized superovulation using a modified 5-d Double Ovsynch protocol with 4 d of decreasing FSH (Folltropin, 400mg/cow) treatments. All animals were flushed between 65 to 75DIM, near peak production (39.6 kg/d). Non-sexed frozen semen (15x106 sperm/straw) were produced from single ejaculates of 2 high-fertility sires and cows were inseminated twice (12h and 24h after final GnRH treatment). To avoid variation, a single batch of FSH was used and 2 experienced AI technicians performed all breedings and flushings, which occurred 6d after synchronized ovulations. In addition, a treatment-blinded technician graded all embryos. The proc GLIMMIX of SAS was used to compare embryo characteristics using cow as a random experimental unit. Site of semen deposit did not change fertilization rate (~78%) or any other measured embryo characteristic, as shown in the table below. In conclusion, placement of semen in uterine horns failed to improve fertilization rates or number of transferable embryos compared with standard body-AI in superovulated, high-producing dairy cows bred twice with high-fertility semen. Supported by Adisseo, Accelerated Genetics, USDA Grant 2010–85122–20612.

Table 1.

Item	Body AI	Horn AI	P-value
n	35	37	
CL number (ultrasound on the day of the flush)	17.1±1.5	17.6±1.3	0.58
Total ova/embryos recovered	7.1±1.0	8.7±1.4	0.33
% Ova/embryos per CL	40.4±5.1	44.4±4.5	0.75
Number of fertilized ova	5.3±0.9	6.6±1.1	0.33
% Fertilized ova	78.5±5.1	78.2±4.7	0.97
Number of transferable embryos	3.9±0.8	5.3±0.9	0.18
% Transferable embryos of fertilized	57.6±6.9	64.3±5.5	0.81
Number of degenerate embryos	1.4±0.4	1.3±0.4	0.90
% Degenerate of fertilized	32.2±6.7	20.7±4.9	0.39

Key Words: superovulation, AI technique, dairy cow

W217   Influence of sex and breed of the calf on synchronization and pregnancy rates in cows submitted to timed AI. A. P. Lemes*[1], R. F. G. Peres[2], A. D. P. Rodrigues[3], M. M. Guardieiro[1], E. Oba[3], G. B. Mourão[1], and R. Sartori[1], [1]University of São Paulo, Piracicaba, SP, Brazil, [2]Agropecuária Fazenda Brasil, Barra do Garças, MT, Brazil, [3]São Paulo State University, Botucatu, SP, Brazil.

Studies have shown that weight, sex, and breed of calves influence the productive status of cows. This study evaluated the influence of sex and breed of calves on the response of cows to a timed-AI (TAI) protocol. Primiparous and multiparous Nelore (*Bos indicus*) cows (n = 865; 44.3 ± 2.1 d postpartum[DPP]) were submitted to the following protocol: D0 - intravaginal progesterone (P4) device (DIB, Syntex S.A., Argentina), 2 mg estradiol benzoate (Gonadiol, i.m., Syntex S.A.) and 12.5 mg dinoprost tromethamine (Lutalyse, i.m., Pfizer Animal Health); D8 - 12.5 mg dinoprost, 0.8 mg estradiol cypionate (ECP, i.m., Pfizer Animal Health), withdrawal of DIB, and 300 IU eCG (Novormon 5000, i.m., Syntex S.A.) or saline; D10 - TAI using frozen/thawed semen from 3 bulls. Ovaries were evaluated by ultrasound (Mindray 2200 VET DP) 7 and 30 d after AI for presence or absence of CL and pregnancy diagnosis. Data were analyzed using PROC GLIMMIX of SAS and the use of eCG on Day 8, body condition score (BCS), DPP, sex (F: Female; M: Male), and breed (C: Crossbred Nelore × Angus; N: Nelore) of calves were included in

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the model. Results are presented as least squares means ± SE. Cows being suckled by F or M and C or N calves had similar synchronization rates (F: 86.2 ± 2.1% vs. M: 86.8 ± 2.1%; P = 0.79; C: 86.1 ± 2.3% vs N: 87.0 ± 2.1%, P = 0.74). Likewise, synchronized cows had no effect of calf sex or breed on conception rate (F: 46.8 ± 5.4% vs. M: 46.3 ± 5.3%; P = 0.90; C: 48.9 ± 5.5% vs. N: 44.2 ± 5.4%; P = 0.33). Cows with a better BCS had higher synchronization and pregnancy rates (P < 0.001). Interestingly, serum P4 concentrations of the synchronized cows were affected by the sex of the calf on d 7 (F: 4.9 ± 0.8 ng/mL vs. M: 4.0 ± 0.8 ng/mL, P = 0.03) and 14 (F: 8.1 ± 1.3 ng/mL vs. M: 6.2 ± 1.3 ng/mL, P = 0.01), and by calf breed (C: 4.9 ± 0.8 ng/mL vs. N: 4.0 ± 0.8 ng/mL, P = 0.04) on d 7 after AI. In conclusion, although there was a relationship between calf sex and breed on circulating P4 of the dam, these variables did not influence the reproductive efficiency of the cow bred by TAI early in postpartum. Supported by SYNTEX S.A. of Argentina, and Agropecuária Fazenda Brasil, CAPES, FAPESP, and CNPq of Brazil.

Key Words: calf, reproduction, artificial insemination

W218   The requirement of GnRH at the onset of the 5-d Select Synch + CIDR program in beef heifers. F. M. Abreu*[1], L. H. Cruppe[1], M. V. Biehl[3], A. D. P. Rodrigues[2], M. D. Utt[1], G. A. Bridges[4], J. L. M. Vasconcelos[2], and M. L. Day[1], [1]*The Ohio State University, Columbus, [2]Sao Paulo State University, Botucatu, SP, Brazil, [3]University of Sao Paulo, Pirassununga, SP, Brazil, [4]University of Minnesota, Grand Rapids.*

The objective of this study was to investigate whether or not the omission of the GnRH injection at CIDR insertion would affect luteolysis and pregnancy rates to AI in the 5-d Select Synch + CIDR program in beef heifers. Prepubertal (n = 27) and cyclic (n = 124) heifers from 3 locations were randomly assigned to receive either 100μg GnRH (n = 77) or 2 mL saline (SAL, n = 74) at CIDR insertion (d −5); followed by 25 mg PGF coincident with CIDR removal 5 d later (d 0). Blood samples were collected on d −15 and −5 to assess puberty status. Estrus detection was performed twice daily from 0 to 60 h after PGF and AI based on the AM/PM rule. Heifers not detected in estrus were timed AI followed by a GnRH injection 72 h after PGF. In 2 locations, blood samples from heifers with no visual signs of estrus were collected at 72 h to assess progesterone concentrations. Ovarian ultrasonography was performed on d 0 to determine ovulation response to the first GnRH (GnRH treatment) and presence of CL in all heifers. Pregnancy diagnosis was performed on d 35. At CIDR withdrawal, presence of a minimum of one CL in the ovaries tended (P = 0.09) to be greater in the GnRH (94%) than SAL (86%) treatment, and was greater (P < 0.01) in cyclic than prepubertal heifers (95 and 67%). Size of the largest ovarian follicle on d 0 was greater (P < 0.01) for heifers in the SAL (10.6 ± 0.4 mm) than GnRH (8.9 ± 0.3 mm) treatment. The proportion of heifers detected in estrus and time to estrus did not differ (72% and 48.2 ± 0.8 h). For the 2 locations in which blood samples were collected from heifers that did not exhibit estrus (n = 27), incidence of luteal regression was 85.2% and did not differ between treatments. All 4 heifers deemed to have not regressed their CL (not detected in estrus and progesterone concentrations >0.5 ng/ml) were in the GnRH treatment. Synchronization pregnancy rate did not differ between GnRH and SAL treatments (48 and 53%, respectively). In conclusion, administration of GnRH at the onset of the 5-d Select Synch + CIDR protocol did not influence the incidence of luteal regression or benefit pregnancy rates to AI in beef heifers.

Key Words: GnRH, beef heifers, pregnancy rate

W219   Efficacy of the "CoPGF" approach to induce luteolysis in the 5-d CO-Synch + CIDR program in lactating beef cows. M. V. Biehl*[1,3], L. H. Cruppe[1], F. M. Abreu[1], A. D. P. Rodrigues[4], M. L. Mussard[1], G. A. Bridges[2], A. V. Pires[3], and M. L. Day[1], [1]*The Ohio State University, Columbus, [2]University of Minnesota, Grand Rapids, [3]University of Sao Paulo, Piracicaba, SP, Brazil, [4]Sao Paulo State University, Botucatu, SP, Brazil.*

The objective of this study was to determine the efficacy of using 50 mg (2 25 mg doses; CoPGF) of PGF2α (PGF) administered coincident with CIDR removal in the 5-d CO-Synch + CIDR estrous synchronization program in postpartum beef cows. This approach was compared with treatment with 2 25 mg doses of PGF given at an 8 h interval (25 mg at CIDR removal and 25 mg 8 h later; 8hPGF). Lactating multiparous cows (n = 51) were randomly assigned by age and calving date to treatments (CoPGF, n = 27; 8hPGF, n = 24). Blood samples were collected on d −15 and −5 to assess reproductive status at the onset of the program. All cows received 100 μg GnRH (GnRH-1; Cystorelin) at CIDR insertion (d −5). On d 0, the CIDR was removed 2 h before (h −2) the CoPGF treatment or the initial PGF injection of the 8hPGF treatment was given (h 0). Blood samples were collected at h 0, 8, 16, 24, 36, 60, and 84 to assess circulating progesterone concentrations (P4) and luteal regression, and on d 13 to assess function of the subsequent CL. Ovarian ultrasonography was performed on d −5 and 0 to identify the presence of spontaneously formed and GnRH-induced CL, respectively. Estrus detection was performed twice daily from d 0 to 5. Ovulation to GnRH-1 tended to be greater (P = 0.07) for the 8hPGF (83.3%) than the CoPGF group (59.3%). Proportion of animals in anestrus at CIDR insertion (33.3%) and presence of at least one CL at CIDR removal (94.1%) did not differ between treatments. The proportion of cows detected in estrus and interval from CIDR removal to estrus did not differ between treatments (8hPGF, 66.7% and 67.0 ± 2.79 h; and CoPGF, 70.4% and 64.6 ± 2.03 h). The incidence of luteolysis did not differ between treatments (8hPGF, 95.2%; CoPGF, 95.7%) and was characterized by P4 below 1 ng/ml by h 36 and thereafter to h 84. Progesterone concentrations on d 13 did not differ (8hPGF, 2.65 ng/ml; CoPGF, 3.03 ng/ml). In conclusion, the CoPGF approach is a highly effective method to induce luteal regression with the 5-d CO-Synch + CIDR program.

Key Words: beef cows PGF, PGFα, 5-d program

W220   Effects of GnRH and administering number of PGF2α doses in the 5-d timed AI program on ovarian responses and fertility of dairy heifers. F. S. Lima*[1], E. S. Ribeiro[1], R. S. Bisinotto[1], N. Martinez[1], L. F. Greco[1], K. N. Galvão[1], C. A. Risco[1], W. W. Thatcher[1], M. Amstalden[2], and J. E. P. Santos[1], [1]*University of Florida, Gainesville, [2]Texas A&M University, College Station.*

Objectives were to determine if GnRH at the initiation of 5-d timed AI combined with 2 doses of PGF2α improve ovarian responses and pregnancy per AI (P/AI) of dairy heifers. Holstein heifers (n = 2,118) received an intravaginal insert (CIDR) containing progesterone (P4) on d0. Inserts were removed on d5 and heifers were treated with PGF2α (25 mg dinoprost). GnRH was administered on d8 concurrently with timed AI. Heifers were allocated randomly to receive no additional treatment (NG1P = 711), a second injection of PGF2α on d6 (NG2P = 696), or an injection of GnRH on d0 and a second injection of PGF2α on d6 (G2P = 711). Ovaries were scanned on d0 and 5. Blood was sampled at AI to measure P4 concentrations. Pregnancy was diagnosed on d 32 and 60 after AI. Data were analyzed using GLIMMIX of SAS. Administration of GnRH on d0 increased ovulation and the proportion of heifers with a new CL on d5 (Table). The proportion of heifers with a CL on d5 (86.8%) did not differ among treatments. The second injection of PGF2α

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improved luteolysis compared with a single PGF2α despite administration of GnRH on d0. Concentration of P4 at AI was greater for NG1P than NG2P or G2P (0.36 vs. 0.27 vs. 0.30 ng/mL). The P/AI on d 30 and 62 were greater for G2P than NG1P or NG2P, but pregnancy loss did not differ with treatment (NG1P = 7.4%, NG2P = 6.3% and G2P = 4.3%). Heifers with a new CL on d5 that received 2 PGF2α had greater P/AI than those with a new CL but that received a single PGF2α (62.8% vs. 45.7%). Combining GnRH with 2 doses of PGF2α in the 5-d timed AI protocol improved fertility because of greater ovulation associated with adequate luteolysis in dairy heifers.

Table 1. Effect of treatment on reproductive responses [% (no./no.)] of dairy heifers

| | Treatment | | | |
	NG1P	NG2P	G2P	P-value
Ovulation d0	13.2 (40/303)[b]	12.8 (38/296)[b]	27.6 (85/308)[a]	0.01
New CL d5	20.5 (62/303)[b]	19.6 (58/296)[b]	33.8 (104/308)[a]	0.01
Luteolysis				
P4<0.5 ng/mL	82.5 (151/183)[b]	92.9 (169/182)[a]	88.3 (158/179)[ab]	0.01
P4<0.3 ng/mL	61.2 (112/183)[b]	74.2 (135/182)[a]	71.0 (127/179)[a]	0.03
Pregnant				
d 32	52.9 (376/711)[b]	55.0 (383/696)[b]	61.7 (439/711)[a]	0.01
d 60	49.0 (348/711)[b]	51.6 (359/696)[b]	59.1 (420/711)[a]	0.01

[a,b,c]$P < 0.05$.

Key Words: dairy heifer, luteolysis, fertility

W221   Comparison between the GGPG and two PGF2α based resynchronization programs on fertility in lactating dairy cows. R. G. S. Bruno*[1,2], A. M. Farias[1], K. J. Lager[1,2], D. E. Hawkins[2], and T. R. Bilby[1], *[1]Texas A&M University, College Station, [2]West Texas A&M University, Canyon.*

The objective was to compare the use of either GnRH or PGF as a presynchronization strategy before a resynchronization program on fertility in dairy cows. Lactating cows (n = 2327) from a dairy in TX were assigned to 1 of 3 resynchronization programs at 29 ± 3 and examined for pregnancy at 36 ± 3 d after AI. Resynchronization programs consisted of: GGPG (n = 458), an injection of GnRH at enrollment followed by the Ovsynch protocol initiated at the time of non-pregnancy diagnose (NPD); POV7 (n = 940) an injection of PGF at NPD followed by the Ovsynch protocol 7 d later; and POV11 (n = 929) injection of PGF 3 d after NPD followed by the Ovsynch protocol 11 d later. Cows were AI at any time during the program and, if AI, cows were removed from subsequent injections. Ovaries were scanned and blood sampled for progesterone (P4) levels on day of first GnRH and PGF of Ovsynch. Pregnancy per AI (P/AI) was diagnosed at 36 and 66 d after AI. Overall 64% of cows were diagnosed pregnant 7 d after enrollment. Among non-pregnant cows, GGPG reduced ($P < 0.01$) re-inseminations upon ED (GGPG = 23.3 vs. POV7 = 74.9 and POV11 = 79.6%). Treatment did not affect overall P/AI (35d, GGPG = 29.2, POV7 = 28.7 and POV11 = 31.9%; 66d, GGPG = 25.8, POV7 = 26.6 and POV11 = 30.2%) or pregnancy loss between 36 and 66 d after AI ($P > 0.41$). Cows AI upon ED had greater ($P = 0.02$) P/AI then cows TAI (ED = 32.3, TAI = 25.1%). However, treatment did not affect ($P > 0.31$) P/AI for cows AI upon ED (35d, GGPG = 29.6, POV7 = 29.4 and POV11 = 35.7%; 66d, GGPG = 27.3, POV7 = 28.1 and POV11 = 33.7%) or TAI (35d, GGPG = 29.1, POV7 = 25.0 and POV11 = 16.9%; 66d, GGPG = 25.3, POV7 = 22.1 and POV11 = 16.9%). Treatment affected ($P < 0.01$) the median days between NPD and re-breeding (GGPG = 10, POV7 = 4 and POV11 = 7 d). At beginning of Ovsynch, more GGPG cows had CL (GGPG = 86.8 vs. POV7 = 50.7 and POV11 = 60.0%, $P < 0.01$). However, treatment did not affect ($P = 0.42$) ovulation to the first GnRH of Ovsynch. In conclusion, GGPG reduced re-insemination during resynchronization. Reproductive program did not affect P/AI but starting the resynchronization with PGF at NPD shortened the interval between non-pregnancy diagnose and re-breeding.

Key Words: dairy cow, fertility, resynchronization

W406   See abstract #92

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